Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 24, pp. 2937-2940, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00540-9

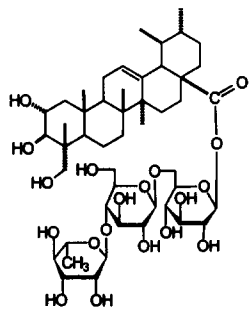
ASIATICOSIDE MIMETICS AS WOUND HEALING AGENT

Pil-Jong Shim, Jae-Ho Park, Min-Sun Chang, Min-Jung Lim, Do-Ha Kim, Young Hoon Jung,[+] Sang-Sup Jew,[++] Eun Hee Park, and Hee-Doo Kim*

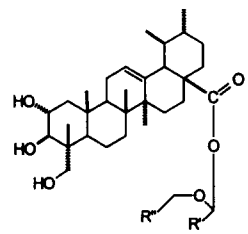
College of Pharmacy, Sookmyung Women's University, Yongsan-Ku, Seoul 140-742, Korea, College of Pharmacy, Sungkyunkwan University,[+] Suwon 440-746, Kyunggido, Korea, and College of Pharmacy, Seoul National University,[++] Kwanak-Ku, Seoul 151-742, Korea

Abstract Novel asiaticoside mimetics simplified the sugar moiety by alkoxyalkyl groups were synthesized, and tested their wound healing effects by tensile strength measurement.

Madecassol®, the titrated extract from *Centella asiatica*, has been used as a wound healing agent and for the prevention of cicatrization.[1] Wound healing process related to collagen synthesis in skin dermis[2] are associated with three pentacyclic triterpenes, asiaticoside, asiatic acid, and madecassic acid.[3] Among them, both asiatic acid and asiaticoside are the essential ingredients for biological activity. Asiaticoside, a few 1-O-acyl-D-glucopyranose found in nature, is a trisaccharide ester of asiatic acid. Although the pharmacological action of asiaticoside has been well documented,[4] the role of sugar in asiaticoside is still unclear. Some data[5] suggest that asiatic acid is the only component responsible for collagen synthesis stimulation and the therapeutic effects of asiaticoside may be mediated through conversion to asiatic acid.



Asiaticoside

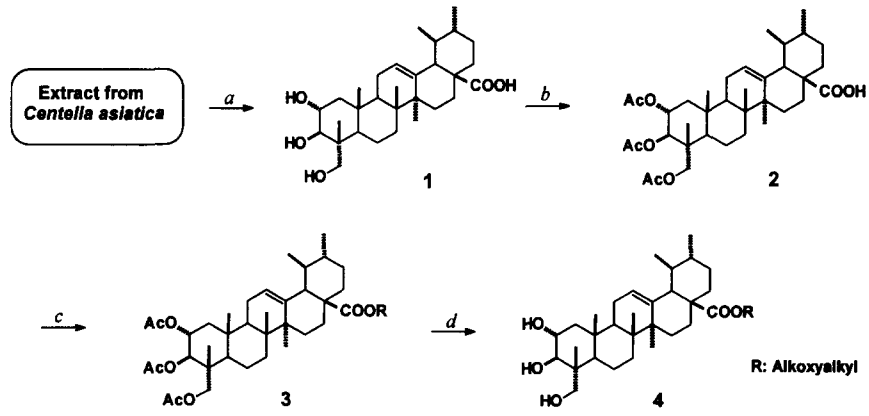


Asiaticoside Mimetic

However, it is well known that glycosides of therapeutic importance having appropriate sugar molecules will be more effective than free aglycone. Even if the sugar moiety in asiaticoside may not be necessary for biological activity, it may be possible to change the efficacy of asiatic acid aglycone by regulating the pharmacokinetic parameters such as absorption, distribution, bioavailability, or therapeutic width.[6] In this sense, the development of asiaticoside analogues may prove useful in the production of more active wound

healing agents as well as lead to information on the asiaticoside pharmacophore. In general, drugs applied to the skin are poorly absorbed, particularly if they contain hydroxyl groups that can interact with the skin or binding site in the keratin. Because the carboxylic and hydroxyl group can inhibit the ease dermal permeation of asiatic acid, they should be changed to suitable forms for topical absorption. Thus, in order to increase dermal permeability and to facilitate the release of asiatic acid after dermal absorption, we decided to design the novel asiaticoside mimetic. Taken into account of the fact that esterases are ubiquitous, and that esters are ease to control the degree of lipophilicity, our preliminary approach to prodrug design begins with simple esterification of hydroxyl and carboxylic group of asiatic acid. However, during the synthesis, we found that simple alkyl esters of carboxylic acid group of asiatic acid are very reluctant to hydrolysis for steric reason. Therefore, the target molecule was designed to mimic the glycosidic bond by conversion of carboxylic group to alkoxyalkyl ester, and thereby to release asiatic acid readily after skin absorption. The new compounds of this study were readily prepared by the route shown in Scheme. Asiatic acid (**1**) was prepared by hydrolysis of the extract[7] from *Centella asiatica* by sodium hydroxide in methanol at reflux temperature, followed by neutralization. This crude asiatic acid was directly acetylated with acetic anhydride in pyridine to give triacetylasiatic acid **2**. The conversion of triacetylasiatic acid **2** to the alkoxyalkyl ester **3** was carried out by treatment with chloroalkyl alkyl ethers and diisopropylethylamine in dichloromethane, or with alkyl vinyl ethers and catalytic amount of pyridinium *p*-toluenesulfonate in dichloromethane. Careful hydrolysis of **3** with potassium carbonate in methanol gave **4** in good yields.

Scheme



Reagents and Conditions: *a*) NaOH, MeOH, reflux; neutralization by aq.HCl; *b*) Ac_2O, pyridine; *c*) chloroalkyl alkyl ether, diisopropylethylamine, CH_2Cl_2, room temperature / *or* alkyl vinyl ether, PPTS, CH_2Cl_2 , room temperature; *d*) K_2CO_3, MeOH

The synthesized compounds were tested their wound healing effect by measuring the tensile strength of skin strips from the wound segments according to the literature method.[8] Statistical analysis of the differences between groups was performed using the Student's *t*-test for comparison of means. Values for *P*

less than 0.05 were considered significant. The wound healing effects for compounds **1**, **3a~f**, and **4a~i** are outlined in Table 1 and Table 2.

Table 1. Effect of asiaticoside derivatives **4** on wound healing in rats at 7th postoperative day.

Compound [a]	Structure (R)	Tensile Strength [b] (g / cm^2)	Relative value
Control		287±12.7	100
Asiatic acid(1)	H	418±39.9*	146
Asiaticoside(4a)	Rham-Glu-Glu	466±40.2**	162
4b	Benzyloxymethyl	327±26.4	114
4c	1-Butoxyethyl	362±30.7	126
4d	1-Ethoxyethyl	387±41.5	135
4e	Methoxyethoxymethyl	398±33.6*	139
4f	Methoxymethyl	404±41.9*	141
4g	Octyloxymethyl	468±26.1**	163
4h	Ethoxymethyl	476±28.3**	166
4i	2-Tetrahydropyranyl	481±27.2*	168

[a] Each group contained 8 or more rats. One skin incision was made in each rats, and treated topically with 50mg of 1% ointment[9] of test compounds once a day during the experimental period. [b] Tensile strength was measured by Rheometer(Fudoh Kogyo, Japan) with digital readout. Each value represents mean ± S.E. * $P< 0.05$, ** $P< 0.01$

As shown in Table 1, wound healing effect of the compound **4** is strongly influenced by R group. Using asiatic acid as a reference point, it can be seen that incorporation of relatively polar and/or bulky R group results in reduced wound healing effect. However, asiaticoside with a very polar and bulky R group was found to have better wound healing effect than asiatic acid. **4i** with bulky R group also shows comparable activity with asiaticoside in potency. Interestingly, these two compounds share the pyranose structure. **4g** and **4h** with relatively less polar and less hindered R showed the enhanced efficacies which are comparable with that of asiaticoside. Next, our attention was drawn to hydroxyl groups of asiatic acid. These hydroxyl groups were reported to be metabolized by oxidation or conjugation,[10] and could be modified to satisfy the optimum lipophilicity for maximal biological activity. Thus, we examined the effect of blocking of hydroxyl group of asiaticoside derivatives by forming an acetate. The results of acetylation are provided in Table 2.

Table 2. Effect of asiaticoside derivatives **3** on wound healing in rats at 7th postoperative day.

Compound	Structure(R)	Tensile Strength (g / cm^2)	Relative value
Control		227±21.2	100
Asiatic acid(1)		336±19.0**	148
3a	Octyloxymethyl	310±30.3	137
3b	Methoxyethoxymethyl	320±32.7*	141
3c	Methoxymethyl	330±32.7*	145
3d	2-Tetrahydrofuranyl	331±25.2*	145
3e	2-Tetrahydropyranyl	393±31.0**	173
3f	Ethoxymethyl	487±31.9**	215

Clearly, acetylation is also sensitive to biological effects. In particular, acetylation of hydroxyl groups of **4g** and **4h** produce the most notable impact on activity; conversion of **4h** to **3f** resulted in greatly enhanced activity,

but opposite effect was observed in the case of **4g**. In this series of compounds, **3f** with ethoxymethyl group at R shows most excellent wound healing effect. The enhanced wound healing effects of **3e**, **3f** and **4i** compared with asiaticoside were also confirmed by wound area method.[11] They gave 20-25% less wound areas on the 7th postoperative day than asiaticoside, which were estimated to be significant based on Student's *t*-test. In addition, they required significantly shorter curing times compared with asiaticoside.[12] From this study, it is speculated that sugar portion of asiaticoside is not the essential pharmacophore for biological activity, but enhance the wound healing effect of asiatic acid in topical application, and could be greatly simplified without loss of biological activity.

In summary, we have prepared a series of asiaticoside derivatives, and tested their wound healing effect by tensile strength measurement. Asiaticoside mimetic described here represents one of very few examples of glycoside mimetic from the simplification of sugar moiety by alkoxyalkyl group, and this approach provides valuable insight for the elaboration of sugar moiety in asiaticoside. Efforts continue further to define the activity of this class of compounds and to discover new wound healing agent.

Acknowledgment

Support from Research Center for New Drug Development(KOSEF-RCNDD) and Ministry of Science and Technology(G7-Project) in Korea is gratefully acknowledged.

References and Notes

1. a) Pointel, J. P.; Boccalon, H.; Cloarec, M.; Ledevehat, Joubert, M. *Angiology* **1987**, 38, 46. b) Poizot, A; Dumez, D. *C. R. Acad. Sci. [D]* **1978**, 286, 789.
2. Bonte, F.; Dumas, M.; Chaudagne, C.; Meybeck, A. *Ann. Pharm. Fr.* **1995**, 53, 38.
3. Polonsky, J.; Sach, E.; Lederer, E. *Bull. Soc. Chim. Fr.* **1959**, 880.
4. a) Lawrence, J. C. *J. Invest. Dermatol.* **1967**, 49, 95. b) Laerum, O. D.; Inversen, O. H. *Cancer Res.* **1972**, 32, 1463. c) Boiteau, P.; Ratsimamanga, A. R. *Therapie* **1956**, 11, 125. d) Boiteau, P.; Niegeon-Dureuil, M; Ratsimamanga, A. R. *Compt. Rend.* **1951**, 760. e) Farris, G. *Minerva Med.* **1960**, 56, 2244. f) Lawrence, J. C. *Eur. J. Pharmacol.* **1967**, 414. g) Ratsimamanga, A. R.; Niegeon-Dureuil, M.; Sodigne, G. C. *Compt. Rend. Soc. Biol.* **1952**, 152, 1110.
5. a) Bonte, F.; Dumas, M.; Chaudagne, C.; Meybeck, A. *Planta Med.* **1994**, 60, 133. b) Rush, W. R.; Murray, G. R.; Graham, D. J. *Eur. J. Drug Metab. Pharmarcokinet.* **1993**, 18, 323. c) Maquart, F. X.; Bellon, G.; Gillerey, P.; Wegrowski, Y.; Borel, J. P. *Connect. Tissue Res.* **1990**, 24, 107.
6. Medda, S.; Das, N.; Bachhawatt, B. K.; Mahato, S. B.; Basu, M. K. *Biotechnol. Appl. Biochem.* **1990**, 12, 537.
7. The extract consist of about 40% asiaticoside, 28% asiatic acid, and 29% madecassic acid.
8. a) Schulte, R.;Domenjoz, R. *Med. Pharmacol. Exp.* **1967**, 16, 453. b) Seki, M.; Aita, H.; Ueki, S.; Yoneta, T.; Takemasa, T.; Hori, Y.; Morita, H.; Chaki, K.Tagashira, E. *Folia Pharmacol. Japon* **1992**, 100, 165.
9. The ointment consist of 30% propylene glycol, 5% white petrolatum, 15% glycol stearate, and 50% water.
10. Chasseaud, L. F.; Fry, D. R.; Hawkins, D. R.; Lewis, J. D.; Sword, I. P.; Taylor, T.; Hathway, D. E. *Arzneim.-Forsch.* **1971**, 21, 1379.
11. Hunt, T. K.; Ehrlich, H. P.; Garcia, J. A.; Dunphy, J. E. *Ann. Surg.* **1969**, 170, 633.
12. Unpublished data.

(*Received in Japan* 9 *September* 1996; *accepted* 6 *November* 1996)